Effects of Tonicity on Tension and Intracellular Sodium and Calcium Activities in Sheep Heart

Mario G. Lado, Shey-Shing Sheu, and Harry A. Fozzard

From the Cardiac Electrophysiology Laboratories, Departments of Medicine and the Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois

SUMMARY. We have measured the effects of changing tonicity of the bathing solution on intracellular sodium and calcium activities and tension of sheep cardiac Purkinje strands and ventricular muscle. For Purkinje strands in solutions of normal tonicity, resting membrane potential was $-77.4 \pm 0.4$ mV (mean $\pm$ se), sodium activity was $7.9 \pm 0.4$ mm, and calcium activity was $98 \pm 9$ nM. For ventricular muscle in solutions of normal tonicity, resting membrane potential was $-86.4 \pm 1.2$ mV, sodium activity was $6.9 \pm 0.5$ mm, and calcium activity was $70 \pm 4$ nM. Reduction of tonicity to 75% of normal in both tissues produced depolarization of a few millivolts, and sodium activity fell almost to the level predicted for simple osmotic dilution. In Purkinje strands, calcium activity fell much more than that predicted for simple osmotic dilution. Twitch contraction was reduced in the hypotonic solution. Increase of tonicity to 150% and 200% caused the resting membrane potential to become more negative. In both tissues, sodium activity increased somewhat less than predicted from simple water movement, and calcium activity increased proportionately much more than sodium activity. The much larger change of calcium activity in both hypo- and hypertonic solutions could be explained by water movement plus the effect of sodium-calcium exchange. In hypertonic solutions, tonic tension was increased, along with the rise in calcium activity; however, the twitch tension was reduced. This reduction of twitch tension may be due to a direct effect of hypertonicity on cross-bridge behavior, as has been reported for skeletal muscle. (Circ Res 54: 576-585, 1984)

THE transmembrane Na$^+$ gradient appears to be an important factor in a number of membrane transport systems, including Na-Ca exchange (Reuter and Seitz, 1968; Baker et al., 1969). Systematic change in the Na$^+$ gradient has been used to investigate the role of the gradient in regulating intracellular Ca$^{++}$ (Sheu and Fozzard, 1982). In small cells like those in heart muscle, the Na$^+$ gradient is most easily influenced by change of extracellular Na$^+$, but one would expect that change of gradient by altering intracellular Na$^+$ could be equivalent. Several experimental interventions that lead to change of intracellular Na$^+$ can cause profound effects on intracellular Ca$^{++}$, including Na-K pump blockade (Sheu and Fozzard, 1982; Bers and Ellis, 1982; Lee and Dagostino, 1982) and alteration of stimulation rate (Lado et al., 1982a).

Change in tonicity of the solution’s bathing tissues causes water movement into or out of cells, thereby diluting or concentrating intracellular ions. We have previously investigated the effects of tonicity on transmembrane potential (Akiyama and Fozzard, 1975) and on intracellular K$^+$ activity ($a_{K}$) (Fozzard and Lee, 1976). Those studies indicated that tonicity changes could alter $a_{K}$ substantially. We report here, the effects of tonicity changes on intracellular Na$^+$ activity ($a_{Na}$) and intracellular Ca$^{++}$ activity ($a_{Ca}$). The expectation was that the results might not be simply predicted by water movement, since $a_{Na}$ is regulated by the Na-K pump, and $a_{Ca}$ is influenced by Na-Ca exchange.

Tonicity is also known to have dramatic effects on tension development in muscle (Hodgkin and Horowicz, 1957; Chapman, 1978). Some of these effects might be related to changes in $a_{Na}$ and consequent changes in $a_{Ca}$ (Langer, 1968). We monitored twitch and tonic tension effects of the experimental solutions for possible correlation with measurements of $a_{Na}$. A preliminary report of this work has been made (Lado et al., 1982b).

Methods

Purkinje strands and trabecular muscles were obtained from the left ventricles of sheep hearts, which were transported from the slaughterhouse to the laboratory in cooled oxygenated Tyrode’s solution. The strands of muscles were pinned in a tissue chamber and superfused with solutions at a rate of 4 ml/min and at a temperature of 35 $\pm$ 1°C. The composition of the normal Tyrode’s solution (NT) is shown in Table 1. When gassed with 95% O$_2$ and 5% CO$_2$, the pH was 7.3. Sucrose was used to vary the tonicity of the solutions, while keeping the ionic strength constant. We expected that sucrose would enter cells sufficiently slowly that, during that 10- to 15-minute exposure to test solutions, the change in cell volume would be constant. To make solutions that were either hypo- or hypertonic without change in Na$^+$, it was necessary to lower the Na$^+$ concentration in the control solution (1T) to 116.2 mm, while maintaining the osmolality at 298
mOsm by addition of sucrose. Table 1 shows the composition of this IT solution, and of the hypotonic (1.5T and 2.0T) solutions. The osmolalities of the solutions were measured with an osmometer (G-66, Fiske).

Conventional microelectrodes were made from borosilicate glass (WPI - TW150F), using a vertical puller. Those used for voltage recording were filled with 3 M KCl and had resistances of 5–10 MΩ. The micropipettes used for $a_{\text{Na}}$ recording were the same, but those for $a_{\text{Ca}}$ recording had a somewhat larger tip size. Pipettes used to make ion-selective microelectrodes (ISE) were exposed to the vapors of trichloromethylsilane and dried in an oven at 150°C for 1 hour. They were then filled from the tip by suction with Na+- and Ca++-sensitive microelectrodes was in solutions of pCa between 2 and 8, buffered by ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA) between pCa levels of 4 and 8 (using an apparent stability constant of 3.26 $\times 10^{6}$ M$^{-1}$).

These solutions also contained 10 mM NaCl, 140 mM KCl, and 1 mM MgCl$_2$ and were buffered with imidazole to pH 7.0. More details of electrode preparation and calibration can be found in Sheu and Fozzard (1982), and the value for $a_{\text{Ca}}$ was also higher. The difference in $a_{\text{Ca}}$ is significant at the 1% level by unpaired t-test, but the difference in $a_{\text{Na}}$ does not achieve significance at the 5% level. Sheu and Fozzard (1982) also saw a similar difference in $a_{\text{Na}}$ between resting Purkinje fibers and ventricular muscle, but the difference also was not significant at the 5% level. Calculation of the ratio of Ca$^{++}$ and Na$^{+}$ electrochemical gradients in normal Tyrode’s solution, using these values and the relationship:

$$ n = \frac{2(E_{C_a} - V_m)}{(E_{Na} - V_m)} $$

yielded ratios of 2.60 and 2.55.

**Low Na$^+$, Isotonic Solution**

The IT solution contained 116.2 mM Na$^+$ (90 mM $a_{\text{Na}}$), so we expected that the change from NT to IT solution would hyperpolarize the membrane and lead to somewhat lower $a_{\text{Na}}$ and $a_{\text{Ca}}$ and increased contraction (Sheu and Fozzard, 1982). If $a_{\text{Ca}}$ should fall linearly with reduction in passive Na$^+$ leak, the fall in $a_{\text{Na}}$ would be 22%. We observed, in both Purkinje strands and ventricular muscle, a small hyperpolarization of 1–2 mV (Tables 3 and 4). $a_{\text{Na}}$ fell in Purkinje strands from 7.9 ± 0.4 to 6.9 ± 0.3 mOsm/kg, and in ventricular muscle from 6.9 ±

**Results**

**Normal Tyrode’s Solution**

The values for membrane potential ($V_m$), $a_{\text{Na}}$, and $a_{\text{Ca}}$ that were obtained in normal Tyrode’s solution in Purkinje strands and ventricular muscle are shown in Table 2. The average resting value for $a_{\text{Na}}$ is somewhat higher in Purkinje strands than in ventricular muscle, as previously reported from this laboratory (Sheu and Fozzard, 1982), and the value for $a_{\text{Ca}}$ was also higher. The difference in $a_{\text{Ca}}$ is significant at the 1% level by unpaired t-test, but the difference in $a_{\text{Na}}$ does not achieve significance at the 5% level. Sheu and Fozzard (1982) also saw a similar difference in $a_{\text{Na}}$ between resting Purkinje fibers and ventricular muscle, but the difference also was not significant at the 5% level. Calculation of the ratio of Ca$^{++}$ and Na$^{+}$ electrochemical gradients in normal Tyrode’s solution, using these values and the relationship:

$$ n = \frac{2(E_{C_a} - V_m)}{(E_{Na} - V_m)} $$

yielded ratios of 2.60 and 2.55.

**Low Na$^+$, Isotonic Solution**

The IT solution contained 116.2 mM Na$^+$ (90 mM $a_{\text{Na}}$), so we expected that the change from NT to IT solution would hyperpolarize the membrane and lead to somewhat lower $a_{\text{Na}}$ and $a_{\text{Ca}}$ and increased contraction (Sheu and Fozzard, 1982). If $a_{\text{Ca}}$ should fall linearly with reduction in passive Na$^+$ leak, the fall in $a_{\text{Na}}$ would be 22%. We observed, in both Purkinje strands and ventricular muscle, a small hyperpolarization of 1–2 mV (Tables 3 and 4). $a_{\text{Na}}$ fell in Purkinje strands from 7.9 ± 0.4 to 6.9 ± 0.3 mOsm/kg, and in ventricular muscle from 6.9 ±
FIGURE 1. Panel A: Na⁺-sensitive microelectrode calibration curves. The electrode potential is plotted against Na⁺ activity. Various background concentrations in the calibrating solution are used to simulate intracellular K⁺ concentrations at 0.75T, 1T, 1.5T, and 2T. After subtraction of resting membrane potential, the aN was obtained from curve (○) at 0.75T, curve (●) at 1T, curve (▲) at 1.5T, and curve (▼) at 2T. Panel B: Ca²⁺-sensitive microelectrode calibration curve. The electrode potential is plotted against Ca²⁺ activity. After substitution of resting membrane potential, the aC was obtained from curve (○) at 0.75T, curve (●) at 1T, curve (▲) at 1.5T, and curve (▼) at 2T.
TABLE 2
Control Measurements in Normal Tyrode’s Solution

<table>
<thead>
<tr>
<th></th>
<th>Purkinje strands</th>
<th>Ventricular muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_m$ (mV)</td>
<td>$-77.4 \pm 0.4^*$ (26)†</td>
<td>$-86.4 \pm 1.2$ (8)</td>
</tr>
<tr>
<td>$a_{Na}$ (mM)</td>
<td>$7.9 \pm 0.34$ (26)</td>
<td>$6.9 \pm 0.5$ (8)</td>
</tr>
<tr>
<td>$E_{Na}$ (mV)</td>
<td>$+71.5$</td>
<td>$+75.1$</td>
</tr>
<tr>
<td>$a_{Ca}$ (mM)</td>
<td>$98 \pm 9(25)$</td>
<td>$70 \pm 4(3)$</td>
</tr>
<tr>
<td>$E_{Ca}$ (mV)</td>
<td>+116</td>
<td>+120.4</td>
</tr>
<tr>
<td>$n$†</td>
<td>2.60</td>
<td>2.55</td>
</tr>
</tbody>
</table>

* Mean ± se.
† Number of tissues studied.
‡ See Equation 1 for definition.

0.5 to 5.6 ± 0.6 mM (19% fall). These changes occurred over 2–3 minutes. No significant change in $a_{Ca}$ could be measured, but contraction strength did increase. For these experiments and those that follow, the changes reported to be significant were tested by paired $t$-tests, and yielded values of $P \leq 0.05$. In addition, all changes were reversible, with return to control levels.

Hypotonic Solution

The expected change in $a_{Na}$ upon exposure to the solution with reduced osmolarity (0.75T) if the cells behaved as perfect osmometers is a fall of 23% (not 25%, because of adjustment of the activity coefficient to compensate for the lower internal ionic strength). We would also expect a depolarization because of dilution of $a_k$. Figures 2 and 3 illustrate the observed changes in $V_m$, $a_{Na}$, and $a_{Ca}$ in experiments on Purkinje strands and ventricular muscle. In Purkinje strands, there was the expected depolarization of about 3–4 mV. $a_{Na}$ fell to 5.6 ± 0.5 mM (19% fall), and $a_{Ca}$ fell to 67 ± 22 nM (33% fall) (Table 3). Ventricular muscle depolarized 3–4 mV (Table 4) upon exposure to 0.75T solution, and $a_{Na}$ fell to 4.3 ± 0.5 mM (23% fall). We were not able to obtain satisfactory measurements of $a_{Ca}$ in ventricular muscle in 0.75T solution. No change in resting tension could be seen, but contraction fell by about 30%. All of the effects of hypotonic solutions were readily reversible.

TABLE 3
Sheep Purkinje Strands

<table>
<thead>
<tr>
<th>Tonicity</th>
<th>$V_m$ (mV)</th>
<th>$a_{Na}$ (mM)</th>
<th>$a_{Ca}$ (mM)</th>
<th>$E_{Na}$ (mV)</th>
<th>$E_{Ca}$ (mV)</th>
<th>$n$†</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% (1T)</td>
<td>$-79.0 \pm 0.3^*$ (26)†</td>
<td>$6.9 \pm 0.3$ (36)</td>
<td>$101 \pm 11(25)$</td>
<td>68.6</td>
<td>115.5</td>
<td>2.63</td>
</tr>
<tr>
<td>75% (0.75T)</td>
<td>$-75.9 \pm 1.0$ (8)</td>
<td>$5.6 \pm 0.5$ (8)</td>
<td>$67 \pm 22(5)$</td>
<td>74</td>
<td>121.0</td>
<td>2.58</td>
</tr>
<tr>
<td>150% (1.5T)</td>
<td>$-82.2 \pm 0.6(10)$</td>
<td>$9.5 \pm 1.2$ (10)</td>
<td>$193 \pm 20(10)$</td>
<td>60.0</td>
<td>106.8</td>
<td>2.66</td>
</tr>
<tr>
<td>200% (2T)</td>
<td>$-84.2 \pm 0.5$ (7)</td>
<td>$10.8 \pm 0.3$ (7)</td>
<td>$412 \pm 43(6)$</td>
<td>56.6</td>
<td>96.7</td>
<td>2.60</td>
</tr>
</tbody>
</table>

* Mean ± se.
† Number of tissues studied.
‡ See Equation 1 for definition.

TABLE 4
Sheep Ventricular Muscle

<table>
<thead>
<tr>
<th>Tonicity</th>
<th>$V_m$ (mV)</th>
<th>$a_{Na}$ (mM)</th>
<th>$a_{Ca}$ (mM)</th>
<th>$E_{Na}$ (mV)</th>
<th>$E_{Ca}$ (mV)</th>
<th>$n$†</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% (1T)</td>
<td>$87.5 \pm 1.2^*$ (8)†</td>
<td>$5.6 \pm 0.6$ (8)</td>
<td>$70 \pm 11(3)$</td>
<td>74.2</td>
<td>120.4</td>
<td>2.58</td>
</tr>
<tr>
<td>75% (0.75T)</td>
<td>$-84.3 \pm 1.4$ (6)</td>
<td>$4.3 \pm 0.5$ (6)</td>
<td>$236 \pm 28(3)$</td>
<td>81.2</td>
<td>104.0</td>
<td>2.46</td>
</tr>
<tr>
<td>150% (1.5T)</td>
<td>$-90.8 \pm 1.6$ (6)</td>
<td>$7.1 \pm 0.7$ (6)</td>
<td>$67.8</td>
<td>64.3</td>
<td>64.3</td>
<td></td>
</tr>
<tr>
<td>200% (2T)</td>
<td>$-95.4 \pm 3.7$ (3)</td>
<td>$8.1 \pm 0.5$ (3)</td>
<td>$64.3$</td>
<td>64.3</td>
<td>64.3</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± se.
† Number of tissues studied.
Hyperpolarization was seen in Purkinje cells (3–5 mV) (Table 3). $a_k$ in Purkinje cells rose to $9.5 \pm 1.2$ mM in 1.5T solution (37% increase) and $10.8 \pm 0.3$ mM in 2.0T solution (57% increase). Examples of these effects are illustrated in Figures 4 and 5. In 1.5T solution, $a_{Ca}$ rose to $193 \pm 20$ nM (91% increase) and in 2.0T solution it rose to $412 \pm 43$ nM (400% increase). These changes developed rapidly and were completely reversible. Whereas it often appeared that the change in $a_{Ca}$ developed faster than the change in $a_k$, the location of the electrodes in different cells makes direct comparison of time courses unwise. Resting tension rose in 1.5T solution...
by 32% and in 2.0T solution by 52%. Contraction size fell to 71% and 26%, respectively (Fig. 6).

Ventricular cells showed somewhat more hyperpolarization (4–8 mV) (Table 4). $a_{Ca}$ rose to 7.1 ± 0.7 mm in 1.5T solution (27% increase) and to 8.1 ± 0.5 mm in 2.0T solution (45% increase). These changes are illustrated in Figures 7 and 8. We were successful in monitoring $a_{Ca}$ only during the change to 1.5T solution, where it rose to 23.6 ± 28 nm (330% change). Resting tension rose 38% in 1.5T and 45% in 2.0T solutions. On the other hand, contraction size fell to 26% and 6% of control in the two solutions. The contractile changes were also reversible (Fig. 9).

**Na** and **Ca** Electrochemical Gradient Ratios

Since changing tonicity altered $a_{Na}$ and consequently the Na** electrochemical gradient, the Ca** electrochemical gradient would be expected to change because of the Na-Ca exchange process. Our previous studies (Sheu and Fozzard, 1982) have indicated values of 2.5–2.6 for the apparent coupling ratio during change in Na**, Ca**, V**, and Na. The data obtained in these experiments gave the same coupling ratios in solutions of various tonicity (Tables 2–4). Although there was only a small change in Na** electrochemical gradient (about 7 mV at 2.0T), the change in Ca** electrochemical gradient was much larger (about 27 mV at 2.0T), owing to the nonlinear properties of Na-Ca exchange.

**Discussion**

There are three interesting and important aspects to the results of these experiments. First, the normal values of $a_{Na}$ and $a_{Ca}$ add to the small number of measurements of these intracellular ions available, and the responses to tonicity changes reflect intrinsic cell behavior. Second, the alteration in $a_{Na}$ by water movement provides a test of the hypothesis that the Na** gradient controls $a_{Ca}$ by determining the relation between the Na** electrochemical gradient before

---

**Figure 6.** Panel A: the tension development of a cardiac Purkinje strand in 1.5T solution. NT: normal Tyrode; 1T: low Na, isotonic solution. The upper traces are brief recordings at a faster recording speed, taken at the points indicated. Panel B: the tension development of a cardiac Purkinje strand during exposure to and recovery from 2.0T solution.
and after the interventions. Third, the effects of
tonicity (internal ionic strength) on contraction, and
the possible relationship to $a^{\infty}_N$, offer some infor-
mation about control of contraction in heart muscle.
These three interrelated aspects will be discussed
individually.

Resting Levels of $a^{\infty}_N$ and $a^{\infty}_C$

Ion-sensitive microelectrodes have only recently
become available for measurement of $Na^+$ and $Ca^{++}$
in heart muscle. The first measurements of $a^{\infty}_N$, in
mammalian ventricular muscle were reported by Lee
and Fozzard (1975) to be about 6 mM, and levels
subsequently obtained in various cardiac tissues agree rather closely with that value (e.g., Ellis, 1977;

In experiments already reported, we (Sheu and
Fozzard, 1982) compared ventricular muscle and
Purkinje strands and suggested that $a^{\infty}_N$ might not
be the same in these two tissues. Under conditions
of these experiments, $V_m$ is less negative in Purkinje
strands, and it seems likely that resting Purkinje
cells are farther away from their $K^+$ equilibrium
potential (Lee and Fozzard, 1975; Sheu et al., 1980).
If we assume that this is because resting $P_N$ is higher
in resting Purkinje cells, then resting inward leak of
$Na^+$ would be greater, and consequently the steady
level achieved by the Na-K pump would be higher.
The fact that in Purkinje strands $a^{\infty}_N$ is reduced by
exposure to tetrodotoxin (Deitmer and Ellis, 1980)
supports the idea that resting Purkinje strands are
relatively depolarized because of a higher $P_N$. A
difference in $a^{\infty}_N$ between the tissues would also
predict a difference in $a^{\infty}_C$, if $Na-Ca$ exchange is im-
portant in setting the resting level of $a^{\infty}_C$. Our results
are consistent with this prediction, but the differ-
ences are too small to attribute this with certainty to
$Na-Ca$ exchange. We may also speculate that the
higher resting $a^{\infty}_N$ may underlie the greater sensitiv-
ity of conducting tissue to toxicity by cardiac glyco-
sides (Vassalle et al., 1962), which is thought to be
related to elevated $a^{\infty}_C$ levels (Kass et al., 1978;
Colquhoun et al., 1981).

The levels of $a^{\infty}_C$ in both Purkinje strands and
ventricular muscle are somewhat less than 100 nM.
Using the activity coefficient of 0.32 for solutions of
normal ionic strength, this represents $[Ca^{++}]$, of 0.3
$\mu M$. These values agree with our previous measure-
ments (Sheu and Fozzard, 1982; Lado et al., 1982a)
and with those of other investigators (for review,
see Lee, 1981). The small differences in reported
$\alpha_C$ levels are almost entirely explained by use of
slightly different apparent stability constants for Ca-
EGTA or different temperature for the measure-
ments. All of these measurements are based on the
ISE, and use a variation of the resin ETH 1001, so
systematic errors fundamental to the ISE method
might exist in all of the measurements reported.
However, similar values ($[Ca^{++}]$ = 2.9 \pm 0.3 \times
$10^{-7}$M) have been reported from measurements of
the resting aequorin luminescence in cardiac Pur-
kinje fibers (Weir and Hess, 1984).

Low-Sodium Isotonic Solution

In order to maintain $Na^+$ constant through the
tonicity changes, it was necessary to lower $Na^+$ by
23%. Exposing the tissues to the solution of lower
$Na^+$ caused a fall in $a^{\infty}_N$, as previously reported (Ellis,
1977; Sheu and Fozzard, 1982). Although contrac-
tion strength increased, the measurements in these
experiments failed to show a significant change in
$a^{\infty}_C$. Sheu and Fozzard (1982) previously demonstra-
Lado et al. / Effects of Tonicity on $a_Na$ and $a_{Ca}^+$ in Heart Cells

The reduction in Na$^+$ gradient in the present experiments was quite small, so that a combination of a decreased slope of the Ca$^{++}$ electrode response, hyperpolarization, and the small decrease of $a_{Na}$ may have made a small change in $a_{Ca}^+$ difficult to document here.

**Hypotonic Solution**

Entry into the hypotonic solution was promptly followed by a fall in $a_{Na}$ and $a_{Ca}$. The change in $a_{Na}$ in Purkinje strands was 19%, not significantly different from the 23% predicted for simple osmotic dilution. The $a_{Na}$ change in ventricular muscle was exactly 23%. As mentioned earlier, the expected change was not 25%, because osmotic and chemical activity coefficients are not the same. The increase in activity coefficient as a result of lower ionic strength accounts for the difference. In this range, it appears that the cell is a good osmometer and that neither the Na-K pump nor any other factor except dilution affects $a_{Na}$. It is rather surprising that the fall in $a_{Na}$ produced by dilution was not adjusted to its previous level by action of the Na-K pump. Perhaps the concomitant dilution of $a_k$ influenced the inner pump site affinity for $a_{Na}$. Schümpelri et al. (1982) have reported that osmotically induced changes in $a_{Na}$ in frog skeletal muscle were also predicted by the water movement, without any apparent secondary compensation by the Na-K pump. Whereas the time of exposure to solutions of different tonicities was only 5–10 minutes, the apparent time constant for action of the Na-K pump is 1.5–2.0 minutes (Ellis, 1977; Eisner et al., 1981; Cohen et al., 1982). Therefore, there should have been sufficient time for response by the Na-K pump.

$a_{Ca}^+$ measurements in hypotonic solution could be obtained only in Purkinje strands. The fall exceeded that expected from dilution. The tissues depolarized an average of 3 mV in hypotonic solutions, as expected from dilution of $a_k$ (Fozzard and Lee, 1976; Akiyama and Fozzard, 1975).

**Hypertonic Solutions**

Purkinje strands and ventricular muscle showed similar responses to hypertonic solutions. $a_{Na}$ increased somewhat less than predicted for simple osmotic effect. This modest blunting of the $a_{Na}$ change could result from some stimulation of the Na-K pump, but it is also about the same effect seen previously for $a_k$ response to hypertonic solutions (Fozzard and Lee, 1976). In this previous study, we suggested that the cellular ionic strength (and activity coefficients) might not be estimated accurately under these conditions because of the complex polyanion nature of the cytoplasm. We cannot distinguish at this time between these two alternatives. $a_{Ca}^+$ rose dramatically in hypertonic solution, far
more than expected from simple osmotic concentration. The Na-Ca exchange hypothesis would predict that the increase in $a_{\text{Ca}}$ would lead to a secondary rise in $a_{\text{Ca}}$. Estimation of the apparent coupling ratios under these new conditions showed that Ca$^{2+}$ electrochemical gradients change in proportion to the Na$^+$ electrochemical gradients, as would be expected if Na-Ca exchange controlled the level of $a_{\text{Ca}}$. It must be emphasized that the coupling ratio calculation from the two gradients depends on $V_{\text{m}}$, $a_{\text{Na}}$ and $a_{\text{Ca}}$, all of which contain certain measurement error. Consequently, the constant coupling ratios found in these experiments indicate that the dominant role of Na-Ca exchange in setting $a_{\text{Ca}}$ is plausible, but they do not prove it. We have discussed elsewhere in greater detail the validity of this apparent coupling factor for Na-Ca exchange (Sheu and Fozzard, 1982).

It seems unlikely that the tissues were damaged by the solution changes, since ion activities and contractions returned rapidly to normal after washout of the test solutions. In particular, the tissues survived elevated $a_{\text{Ca}}$ levels ($[\text{Ca}^{2+}] > 1 \mu\text{M}$) without apparent cellular uncoupling or residual damage.

**Tension Responses to Tonicity Changes**

Resting tension was not altered by the Tyrode's solution with reduced Na$^+$, or by the hypotonic solution. However, tonic tension developed quickly in hypertonic solutions, with approximately the same time course as change in contraction strength and the change in $a_{\text{Ca}}$. We have reported that tonic tension often developed when $a_{\text{Ca}}$ reached 200 nm ($[\text{Ca}] > 0.6 \mu\text{M}$), when $a_{\text{Ca}}$ was raised by lowering $a_{\text{Na}}$ in unstimulated fibers, and we suggested that this might represent tension threshold for Ca$^{2+}$ (Sheu and Fozzard, 1982). In these experiments, the tonic tension was also associated with elevated $a_{\text{Ca}}$ of about 200 nm or more. No tonic relaxation was seen in hypotonic solutions, even though $a_{\text{Ca}}$ fell. If tension threshold is not exceeded under conditions, then no relaxation would be expected from the decrease in $a_{\text{Ca}}$. It is reasonable to suggest that the tonic tension produced by hypertonic solutions in these experiments is the result of these steady $a_{\text{Ca}}$ levels.

We (Sheu and Fozzard, 1982; Lado et al., 1982a) have reported good correlation between $a_{\text{Ca}}$ measurements and twitch contraction when the contraction size is modified by low Na$^+$ solutions or by varying the rate of trains of action potentials. This same relationship can also be seen in hypotonic solution, with a comparable fall in $a_{\text{Ca}}$, and contractions. However, in hypertonic solution, the contractions become small in spite of elevated $a_{\text{Ca}}$. This result differs from studies in frog atrial muscle by Chapman (1978), but agrees with the results in other tissues, as reported by Hermanseyer et al. (1972), Chapman and Leoty (1974), and Kawata and Kawagoe (1975).

In skeletal muscle, hypertonic solution also causes a reduction in twitch (Hodgkin and Horowicz, 1957; Gordon and Godt, 1970). Direct Ca$^{2+}$ injection (April et al., 1968) or exposure of skinned fibers to Ca$^{2+}$ solution (Gordon et al., 1973) also produces less tension. These investigations have suggested that the high ionic strength produced by hypertonic solution directly interferes with contraction. Such an effect would certainly explain our results with twitch contractions. However, we have suggested that the tonic tension produced by hypertonic solutions is the result of the elevated $a_{\text{Ca}}$. For this to be true, it is necessary that the hypertonic solution still permit Ca$^{2+}$ binding to troponin, but slow the development of force in response to transient Ca$^{2+}$ release. Force-velocity studies in skeletal fiber (Edman and Hwang, 1977) support this idea that exposure to hypertonic solutions interferes with cross-bridge turnover. However, we cannot rule out additional effects of the hypertonic solutions or the high internal ionic strength on Ca$^{2+}$ release from the sarcoplasmic reticulum, such as may occur in tonic skeletal muscle (Godt et al., 1984).

**Na-Ca Exchange**

Examination of the relationship between the Na$^+$ electrochemical gradient and the Ca$^{2+}$ electrochemical gradient that was begun by Sheu and Fozzard (1982) was extended by these experiments to conditions where $a_{\text{Na}}$ was altered by transmembrane water movement. The results of much larger movements of $a_{\text{Ca}}$ in solutions of various tonicity are consistent with the interpretation that Na-Ca exchange plays an important role in the control of the resting level of $a_{\text{Ca}}$ in heart muscle under a wide variety of conditions. Alternative transport processes to regulate $a_{\text{Ca}}$ include an ATP-dependent sarcoplasmic Ca$^{2+}$ pump, analogous to the Na-K pump (Carroni and Carafoli, 1980; Sulakhe and St. Louis, 1980). The possibility that this alternative transport system has a role in regulating resting $a_{\text{Ca}}$ cannot be ruled out by experiments such as those reported here. However, if they are responsible for these results seen here, then the alternative transport systems would require a complicated dependence on Na$^+$.

---

We appreciate the advice of Dr. Robert Godt.

Supported by Grant HL-20592 from the National Institutes of Health.

Dr. Lado's present address is: Department of Physiology, Faculty of Medicine, University of the Republic, Montevideo, Uruguay.

Dr. Sheu's present address is: Departments of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, New York 14620.

Address for reprints: Dr. H. A. Fozzard, Box 249, University of Chicago Hospitals, 950 E. 59th Street, Chicago, Illinois 60637.

Received December 5, 1983; accepted for publication February 23, 1984.

**References**

Akiyama T, Fozzard HA (1975) Influence of potassium ions and osmolality on the resting membrane potential of rabbit ventric-
Fozzard HA, Lee CO (1976) Influence of changes in external potassium and chloride ions on membrane potential and intracellular potassium ion activity in rabbit ventricular muscle. J Physiol (Lond) 256: 663–689

INDEX TERMS: Sheep heart • Intracellular ions • Hypertonic effects • Cardiac contraction • Ion-sensitive microelectrode

Lee CO, Fozzard HA (1975) Activities of potassium and sodium in rabbit heart muscle. J Gen Physiol 65: 694–708
Sheu S-S, Korth M, Lahmprop H, Fozzard HA (1980) Intrac- 
Effects of tonicity on tension and intracellular sodium and calcium activities in sheep heart.
M G Lado, S S Sheu and H A Fozzard

Circ Res. 1984;54:576-585
doi: 10.1161/01.RES.54.5.576

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/54/5/576

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/